DOI: 10.22319/rmcp.v17i3.7010
Vol. 17(3) | 2026 | e1737010

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# Effects of adding two yeasts to an antibiotic-free diet on some aspects of the digestive function in newly weaned piglets

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## Abstract

A total of 40 Fertilis 20×G Performance piglets aged 19.8 ± 1.56 d and weighing 6.5 ± 0.80 kg were used and assigned to four diets: basal diet without antibiotics (C-) and basal diet with antibiotics (Ab), or *S. cerevisiae* (Sc), or *S. boulardii* (Sb). On d 14 after weaning, five pigs were slaughtered per diet to measure the weight of digestive organs, the activity of pancreatic enzymes, the pH of gastrointestinal contents, and the concentration of volatile fatty acids (VFAs) in the colon. The relative weight of the organs and the pH of the stomach, jejunum, and ileum contents did not vary among the piglets; the pH of the cecum and colon contents was more acidic in animals on the Sc, Sb, and C- diets. The specific activity of the enzymes showed no differences among piglets. The total activity of trypsin and chymotrypsin did not differ among animals, but the amylase activity was higher in piglets on the C- diet, medium in those on the Ab diet, and lower in the others. The two yeasts showed high fermentative and acidifying capacity of the large intestine. The consumption of *S. boulardii* led to higher production of total and unbranched VFAs. Animals on the C- diet exhibited the highest production of branched VFAs. It is concluded that *S. cerevisiae* or *S. boulardii* had similar effects on most of the variables studied; however, the fermentation profile was different, with advantages for *S. boulardii* in the production of unbranched VFAs.

**Keywords:** *S. cerevisiae* and *S. boulardii*; Probiotic; Pigs; Weaning; Enzymatic activity; Volatile fatty acids

Received: December 10, 2025

Accepted: May 14, 2026

Published: September 07, 2026

## Introduction

Antibiotics have been supplemented in diets at subtherapeutic levels as animal growth promoters to correct gastrointestinal tract (GIT) problems and to reduce the incidence of diarrhea for almost 70 yr; nevertheless, in recent years, there has been a growing interest in eliminating or minimizing their use, because they can increase the number of resistant bacterial strains and transfer cross-resistance to other microorganisms, with negative implications for human and animal health[1]. On the other hand, the growing demand for organic products has forced animal nutrition experts to seek natural substitutes that include medicinal plants and beneficial microorganisms. The feed-producing livestock industry possess a wide range of "bioactive" products alternative to antibiotics, including prebiotics, probiotics, and additives of organic acids that contribute greatly to the integrity of the intestinal epithelium and modulate the immune system of animals[2]. There is a growing trend towards the use of safe additives, such as functional and nutraceutical feeds[3].

Probiotics are considered a good alternative to replace antibiotics[4-6]. The yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) has been used as a probiotic in animal diets to reduce the use of growth-promoting antibiotics, because of its positive effects on productive indicators, gut microbiota, and health[7]. Beneficial effects of adding *Saccharomyces boulardii* (*S. boulardii*) to diets for weaned piglets have also been observed on the stimulation of the immune system in a different manner from *S. cerevisiae*[8]. The species *Boulardii*[9] is effective against pathogenic bacteria, reduces inflammation caused by diseases in the GIT[8], and promotes the intestinal health in piglets[10]. Its probiotic activity has been elucidated as a conjunction of multiple pathways, which include the improvement of intestinal barrier function, competitive exclusion of pathogens, production of antimicrobial peptides, immune modulation, and trophic effects[11].

*S. boulardii* is a tropical yeast strain very similar to *S. cerevisiae*, but different in many of its taxonomic, metabolic, and genetic characteristics[12]. *S. boulardii* is genetically similar to the yeast *S. cerevisiae*, and its classification as a different species or variant of *S. cerevisiae* has been widely discussed[11]. The question posed in this work is whether, when added to antibiotic-free starter diets, the differences between the two yeasts affect some morphophysiological traits of newly weaned piglets. Therefore, this work aimed to evaluate if the type of *Saccharomyces* added to an antibiotic-free diet for newly weaned piglets differentially affects the weight of digestive organs, the activity of the pancreatic enzymes amylase, trypsin, and chymotrypsin, the pH of gastrointestinal contents, and the concentration of volatile fatty acids in the colon.

## Material and methods

The experimental part was conducted at the experimental swine farm of INIFAP's CENID-Physiology, located in Colón, Querétaro, Mexico. The enzymatic activity and the volatile fatty acid profile were determined in the Animal Nutrition Laboratory of the FCN of the Universidad Autónoma de Querétaro, Mexico.

The protocol was reviewed and approved by the Bioethics Committee of the Autonomous University of Querétaro (78FCN2017). The experiment was caried out following the guidelines of the Official Mexican Standard for the production, care, and use of laboratory animals[13], the guidelines of the Official Mexican Standard on methods for slaughtering domestic and wild animals[14], and the guidelines of the International Guiding Principles for Biomedical Research Involving Animals[15].

### Animals, diets, and experimental treatments

A total of 40 Fertilis 20×G Performance piglets (Genetiporc®, PIC Mexico) aged 19.8 ± 1.56 d and weighing approximately 6.5 ± 0.80 kg were used for 2 wk, following a completely randomized design with 4 treatments and 10 replications per treatment, where each piglet was an experimental unit.

The basal diet was considered as a negative control (C-) because it did not contain yeast or antibiotics (Table 1). The basal diet, at the expense of corn, received 0.8 % of *S. cerevisiae* (Yea-Sacc strain 1026, Alltech, United States), or 0.1 % of *S. boulardii* (Levucell SB, CNCM I-1 079, Lallemand Animal Nutrition, Canada), or 0.05 % of an antibiotic (LincoSpectin: 2.2 g lincomycin, 2.2 g spectinomycin, Zoetis, United States) to constitute the other three experimental diets (Sc, Sb, and Ab, respectively). The diets were isoprotein and isoenergetic and were formulated to meet the nutritional requirements of newly weaned piglets[16]. The dry matter and crude protein[17], neutral detergent fiber[18], and metabolizable energy[19] of the basal diet were determined.

**Table 1.** Ingredients and chemical composition of the basal diet (C-), percentage based on the feed offered

| Ingredients | % | Chemical composition | |
|---|---|---|---|
| Corn | 47.00 | Dry matter, %£ | 89.5 |
| Soybean meal | 15.04 | Crude protein, %£ | 21.8 |
| MFM * | 9.23 | Neutral detergent fiber, %£ | 12.3 |
| Whey | 24.70 | Metabolizable energy, kcal·kg$^{-1}$ Ü | 3,300 |
| Corn oil | 1.49 | | |
| Lysine | 0.25 | | |
| Aminogut ** | 0.80 | | |
| Threonine | 0.10 | | |
| Tryptophan | 0.05 | | |
| DP *** | 0.90 | | |
| Vitamins † | 0.08 | | |
| Minerals ϴ | 0.12 | | |
| Biocholine | 0.04 | | |
| Gustor § | 0.20 | | |

* MFM= Menhaden fish meal. ** Aminogut: L-glutamine and L-glutamic acid (1:1), (Ajinomoto, Japan). *** Dicalcium phosphate. † Vitamins per kilogram of diet: vitamin A 10,200 IU; vitamin D 1,980 IU; vitamin E 60 IU; vitamin K 1.20 mg; choline 967 mg; niacin 36 mg; pantothenate 17 mg; riboflavin 7.2 mg; vitamin B12 38 µg; thiamine 0.3 mg; pyridoxine 0.31 mg; biotin 0.08 mg; folate 0.75 mg. ϴ Minerals per kg of diet: copper 14.4 mg; iodine 800 mg; iron 105 mg; manganese 36 mg; selenium 0.3 mg; zinc 144 mg. § Gustor: sodium butyrate (Norel, Spain). £ Valor analyzed. Ü Value calculated.

The experiment was conducted under conditions of controlled temperature (30 and 28 ± 2 °C during the first and second weeks after weaning, respectively). The pigs were housed in a collective cage 38 cm high, 115 cm wide, and 150 cm long, with a slatted floor. The piglets had *ad-libitum* access to water through a nipple drinker in each cage and to the feed through a hopper-type feeder with six slots.

On d 14 of the experiment, five pigs were slaughtered per treatment[14]. Before slaughter, the pigs were kept fasting for approximately 8 h, with *ad libitum* access to water. The animals were first tranquilized with azaperone (Sural®) 20 mg/kg weight; once the biological samples were obtained, the piglets were slaughtered using an overdose of pentobarbital sodium (Pentotal®).

Then, the abdominal cavity was opened to extract the digestive organs (stomach, liver, pancreas, and small and large intestines), and the pH of the stomachal and intestinal contents was measured using a previously calibrated potentiometer (HANNA® Instruments Mexico). The pancreas was cut into two portions on a cold surface, and the portions were stored at -40 °C until determining the activity of the pancreatic enzymes amylase, trypsin, and chymotrypsin.

Pancreatic amylase activity (IU/mg protein) was determined according to Métais[20]. Trypsin activity (IU/mg protein) was determined according to Reboud *et al*[21]. Chymotrypsin activity (IU/mg protein) was determined according to Bergmeyer[22]. The total protein content of the pancreas was determined using Lowry's[23] technique. The specific activity of each enzyme (IU/mg protein) was obtained by dividing the total activity of the enzyme (IU/g pancreas) by the total protein concentration of the pancreas (mg protein/g pancreas). The international unit (IU) is defined as the amount of enzyme that catalyzes the

conversion of one micromole of substrate per minute. The collected samples of the colon content were frozen at -40 °C to determine volatile fatty acids (VFAs) through gas chromatography in an Agilent 6850 equipment according to Roberts *et al*[24].

## Statistical analyses

The data on the relative weight of digestive organs, pH, enzymatic activity, and VFA concentration were analyzed using a simple classification analysis of variance (ANOVA) for a completely randomized design, after verification of data normality by the (modified) Shapiro-Wilks test and uniformity of variance by the Kolmogorov-Smirnov goodness-of-fit test. Duncan's[25] test was used in SPSS version 23. For all the tests, the experimental unit was a piglet.

## Results

The relative weight of the digestive organs and the pH of the stomach, jejunum, and ileum contents (Table 2) did not vary among the piglets that consumed the different diets. Nonetheless, the pH of the cecum and colon contents of the animals on the antibiotic diet (Ab) was less acidic, whereas the animals on the yeast diets, as well as those of the negative control (C-), had the most acidic contents of cecum ($P$<0.001) and colon ($P$<0.01).

**Table 2.** Effects of consuming the experimental diets on the slaughter weight and the morphophysiological characteristics of the gastrointestinal tract of piglets

| | Diets | | | | | |
|---|---|---|---|---|---|---|
| **Variables** | **Ab** | **Sc** | **Sb** | **C-** | ***P*-value** | **SEM** |
| LW, kg | 8.860 [a] | 7.200 [b] | 7.480 [b] | 7.380 [b] | 0.015 | 0.176 |
| Relative weight, g/kg LW: | | | | | | |
| Pancreas | 1.9 | 1.9 | 1.9 | 2.1 | 0.393 | 0.09 |
| Stomach | 8.6 | 8.7 | 9.4 | 8.6 | 0.621 | 0.25 |
| Liver | 28 | 31 | 31 | 28 | 0.246 | 0.76 |
| Small intestine | 62 | 61 | 56 | 55 | 0.242 | 1.54 |
| Large intestine | 18.6 | 19.2 | 18.8 | 20.7 | 0.455 | 0.48 |
| pH of the content: | | | | | | |
| Stomach | 3.6 | 3.1 | 3.2 | 2.4 | 0.128 | 0.168 |
| Jejunum | 7.0 | 6.6 | 6.8 | 6.7 | 0.160 | 0.065 |
| Ileum | 6.7 | 6.4 | 6.5 | 6.9 | 0.374 | 0.113 |
| Cecum | 6.3 [a] | 5.3 [b] | 5.6 [b] | 5.7 [b] | 0.0002 | 0.053 |
| Colon | 6.4 [a] | 5.6 [b] | 6.0 [b] | 6.0 [b] | 0.003 | 0.061 |
| SA (IU/mg protein): | | | | | | |
| Amylase | 1,059 | 792 | 967 | 1,244 | 0.153 | 66.5 |
| Trypsin | 117 | 150 | 156 | 112 | 0.112 | 7.4 |
| Chymotrypsin | 256 | 337 | 408 | 253 | 0.255 | 30.3 |
| TA (IU/g pancreas): | | | | | | |
| Amylase | 120.684 [ab] | 73.573 [b] | 91.217 [b] | 150.547 [a] | 0.044 | 9.197 |
| Trypsin | 13.156 | 13.954 | 13.257 | 12.441 | 0.514 | 347 |
| Chymotrypsin | 28.679 | 31.121 | 35.579 | 29.931 | 0.745 | 2.335 |

LW= live weight; TA= total activity; SA= specific activity; IU= international units.
[ab] Means with different letters in the same row differ significantly.

The specific activity (SA) of pancreatic amylase did not differ among animals; however, it tended ($P$=0.153) to be lower in the animals that consumed yeasts, especially *S. cerevisiae* (Table 2). The total activity (TA) of pancreatic amylase was higher in the animals on the C- diet, medium in those on the Ab diet, and lower in those that consumed yeasts ($P$=0.044).

The total and specific activities of pancreatic trypsin and chymotrypsin were similar among the animals on the different diets; nevertheless, the specific activity of trypsin tended to be higher ($P$=0.112) in the animals that consumed the yeast diets (Table 2).

The concentration of VFAs in the colon differed among the piglets that consumed the different diets (Table 3). The animals on the Sb diet had the highest ($P$<0.0001) total production of VFAs (TVFAs), followed by those on the Sc, C-, and Ab diets.

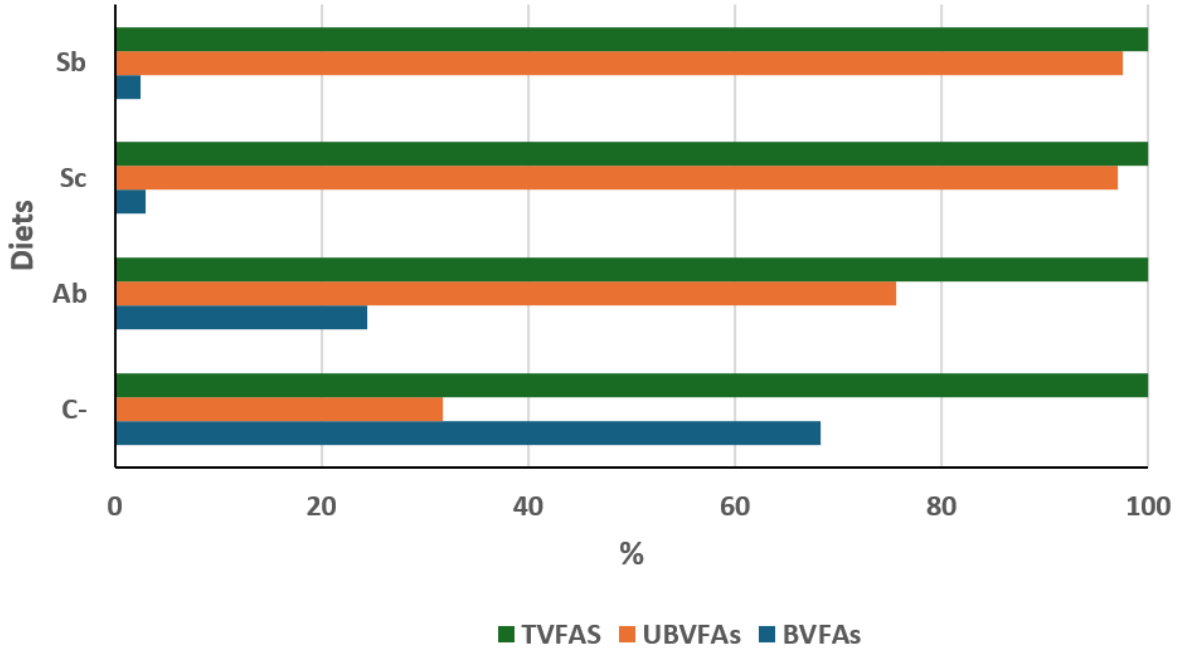
**Table 3.** Profile of volatile fatty acids in the colon contents of piglets, at 14 days post- weaning

| | Diets | | | | | |
|---|---|---|---|---|---|---|
| **VFA (µmol/g)** | **Ab** | **Sc** | **Sb** | **C-** | ***P*-value** | **SEM** |
| Acetic | 33.2 $^{c}$ | 113.4 $^{b}$ | 171.8 $^{a}$ | 26.5 $^{d}$ | 0.0001 | 0.15 |
| Propionic | 10.5 $^{d}$ | 37.7 $^{b}$ | 63.1 $^{a}$ | 14.3 $^{c}$ | 0.0001 | 0.41 |
| Butyric | 13.5 $^{d}$ | 40.8 $^{b}$ | 73.6 $^{a}$ | 14.5 $^{c}$ | 0.0001 | 0.03 |
| Valeric | 5.4 $^{b}$ | 1.5 $^{d}$ | 2.4 $^{c}$ | 12.8 $^{a}$ | 0.0001 | 0.02 |
| Isobutyric | 7.4 $^{b}$ | 2.7 $^{d}$ | 3.4 $^{c}$ | 81.1 $^{a}$ | 0.0001 | 0.07 |
| Isovaleric | 6.5 $^{b}$ | 2.2 $^{d}$ | 2.7 $^{c}$ | 51.2 $^{a}$ | 0.0001 | 0.03 |
| Isocaproic | 6.5 $^{b}$ | 0.9 $^{d}$ | 1.7 $^{c}$ | 14.1 $^{a}$ | 0.0001 | 0.04 |
| UBVFAs | 62.7 $^{d}$ | 193.4 $^{b}$ | 310.9 $^{a}$ | 68.0 $^{c}$ | 0.0001 | 0.46 |
| BVFAs | 20.3 $^{b}$ | 5.9 $^{d}$ | 7.8 $^{c}$ | 146.4 $^{a}$ | 0.0001 | 0.09 |
| TVFAs | 83.0 $^{d}$ | 199.3 $^{c}$ | 318.7 $^{a}$ | 214.4 $^{b}$ | 0.0001 | 0.47 |

UBVFAs= unbranched volatile fatty acids; BVFAs= branched volatile fatty acids; TVFAs= total volatile fatty acids.
$^{ab}$ Means with different letters in the same row differ significantly.

The concentration of UBVFAs was higher in the piglets on Sb and Sc (98 and 97 %, respectively), followed by those on Ab (76 %) and C- (68 %). BVFAs were produced in minimal amounts in the piglets on Sb and Sc (2 and 3 %, respectively) and in higher concentrations in the piglets on Ab and C- (24 and 32 %, respectively) (Figure 1).

**Figure 1.** Percentage composition of VFAs present in the colon content




The UBVFAs most abundant in the colon of the piglets of all diets were acetic acid, butyric acid, and propionic acid (Table 3). The piglets fed the Sb diet exhibited high capacity for acetic acid production ($P$<0.0001) compared to the others. The concentrations of butyric acid and propionic acid followed that of acetic acid and were more abundant in the animals that consumed the two yeasts, being more abundant in the animals on Sb compared to those on Sc ($P$<0.0001). The concentration of valeric acid was higher ($P$<0.0001) in the animals on C-, which did not receive antibiotics or yeasts in their diets, and was very low in those on Sc and Sb.

Regarding BVFAs, the one with highest concentration was isobutyric acid, followed by isovaleric acid and isocaproic acid. The animals that consumed antibiotics (Ab) or yeasts in their diets produced lower amounts of these volatile fatty acids than the animals on C- ($P$<0.0001); in contrast, the animals on yeast diets, especially Sc, showed minimal amounts of BVFAs compared to the others (Table 3).

## Discussion

The highest weight at 14 d after weaning observed in the piglets fed the antibiotic diet (Ab) corroborated its efficiency as a growth promoter, which has been known since 1950[26]. In many studies, the results of the zootechnical behavior of animals that consumed antibiotics are superior compared to alternative compounds. Unfortunately, most of these compounds yield inconsistent results and rarely match the efficacy of antibiotics, thereby showing that there is still no perfect alternative to them[27].

Nonetheless, the benefit observed on the body weight in the piglets on Ab was not transferred to the relative weight of the digestive organs at the post-weaning stage. The weight of the digestive organs was not associated with the presence or absence of antibiotics in the diet, or with the supplementation with the yeasts. Nevertheless, this result suggests that the diets did not exert negative effects on the development of visceral organs. Likewise, other authors[10,28] observed no effect of the consumed diet on the weight of the digestive organs, suggesting that the negative effects of weaning do not affect the weight of the digestive organs, and that the use of dietary nutrients in a period of great stress, such as

the weaning stage, prioritizes maintaining the size of the digestive organs at the expense of the growth of other tissues[29]. The development of viscera is related to the weight and age of piglets[30].

It is interesting to highlight that some of the studied variables showed a positive response to the use of yeasts, and in others, benefits or negative effects were not detected. Likewise, it is observed that the response of these variables is not homogenous between the two yeasts. Variable responses between the two yeasts were also observed by other authors[8], who reported that *S. boulardii* controlled inflammation and preserved the intestinal mucosa more efficiently than *S. cerevisiae*. The differences in the responses to the use of the yeasts are likely due to the genetic differences between them. The genome of *S. boulardii* is very similar to that of *S. cerevisiae*; however, *S. boulardii* has certain specific characteristics that differ significantly from *S. cerevisiae*, both at the genomic and the physiological levels[11,31].

These genetic differences could explain the variations in the physiological responses observed among the animals that consumed the two yeasts in their feed in the present work. In the case of the digestive enzymes studied, reports on the benefits of using live yeasts on the enzymatic activity of the pancreas of newly weaned piglets were not found, and statistically, the actions of the two yeasts had similar effects. Nonetheless, from a biological standpoint, the variation in the enzymatic activity of the pancreas among the animals of the four diet groups observed in the present work could be linked to the variation in feed consumption among the piglets that is observed in this post-weaning phase[32,33], causing greater or lesser presence of substrate in the intestinal lumen; therefore, it can have a significance in the digestion of dietary carbohydrates and proteins. The present results suggest that there was a differentiated modulation of the pancreatic amylase activity, since despite the numerical similarity of the specific activity of this enzyme among the groups that received the two yeasts in their diets, the consumption of *S. cerevisiae* was less efficient in its stimulation. In the case of protease activity, greater stimulation of the pancreas is observed in a similar way in the two groups that consumed both yeasts, but with a greater magnitude than in the piglets on the diets with or without antibiotics; however, this affirmation must be verified.

Yeasts do not have the ability to colonize the GIT[11], and their recovery in the feces does not reach 100 %[34], suggesting that, during their passage through the GIT, they die, and it is possible that their cell walls are used as substrates for the microorganisms existing in the environment, and, therefore, they can function as a prebiotic[35]. One of the mechanisms of action of prebiotics is the stimulation of the exocrine and endocrine secretions of the pancreas[36]. This can likely explain the trend observed in the case of the increased trypsin activity, and perhaps the trend in chymotrypsin activity; nevertheless, it is contradicted by the lower amylase activity in the animals that consumed the yeast-supplemented diets.

More studies are required to verify this affirmation, since in the literature, works relating the enzymatic activity of the pancreas to the addition of the yeasts *S. cerevisiae* and/or *S. boulardii* to the diet of piglets were not found, as has been observed in other animal species. In fish, an increase in the pancreatic amylase activity was observed in larvae fed *S. cerevisiae*[37]. These authors relate this improvement to polyamines (spermidine and spermine) that are present in the yeasts. These polyamines are known to have a trophic effect on the intestinal mucosa of piglets and are important for the maturation of the digestive system, the expression of brush border enzymes, and membrane transport mechanisms[38]. In humans[39], it is mentioned that *S. boulardii* synthesizes and secrets polyamines that participate in cell proliferation and differentiation. In a study conducted using lactating rats treated with spermine[40], it was reported that the activities of amylase, trypsin, and chymotrypsin increased significantly, because it could induce early biochemical and morphological maturation of the exocrine pancreas. The polyamines of the yeasts used in the present study could have also influenced the activity of the pancreatic proteases, which will need to be corroborated.

The fragments of undigested dietary proteins and carbohydrates of endogenous origin that reach the large intestine are fermented by enzymes of microbial origin, generating several products, including VFAs[41]. Acetic acid, butyric acid, and propionic acid are unbranched short-chain fatty acids, which are

produced in the large intestine of mammals by bacteria of the genera *Clostridium*, *Eubacterium*, or *Fusobacterium* through the fermentation of undigested carbohydrates[42]. The concentration of VFAs affects the pH of the large intestine contents[43]. The results of the present work show that the diet consumed played an important role, demonstrating the yeasts' capacity for acidifying the intestinal environment, which is most likely related to the fermentation profile and the increase in UBVFAs[44]. The increase in the concentrations of these VFAs in the treatments with *S. cerevisiae* and *S. boulardii* could have influenced the pH of the colon.

The fermentation products in the piglets' colon found in the present work are indicative of the two yeasts causing differentiated responses in the use of these substrates. The animals fed the diet added with *S. boulardii* showed greater fermentative capacity than those fed *S. cerevisiae*, especially for the formation of UBVFAs, thereby corroborating the findings of other authors[31]. The concentration of the VFAs produced by the microbial fermentation in the colon in the present work shows that the supplementation with the yeasts produced a more desirable profile for the health of the colonic epithelium due to the UBVFAs/BVFAs ratio. UBVFAs serve as an important source of energy for the intestinal wall and provide approximately half of the daily energy requirement of the colonocytes in humans[42,45,46]. In addition to its important energetic contribution, butyric acid presents health benefits, regulating gene expression in the colonocytes and exhibiting immunomodulatory and anti-inflammatory properties[46].

The higher proportion of UBVFAs compared to BVFAs observed in the present work with the consumption of the yeasts suggests a better VFA profile in the animals that consumed the yeasts than in the piglets on Ab and C-, in which there was a higher amount of BVFAs compared to UBVFAs. However, in another study[47], piglets that consumed a diet similar to C- exhibited 98 % production of UBVFAs and 2 % production of BVFAs.

There were other benefits caused by UBVFAs to the animal body[41]. Acetic acid is transported through the blood from the colon to the liver, where it is used by up to 70 %. It is an energy source for hepatocytes, but it is also necessary for the formation of cholesterol and long-chain fatty acids. The remaining 30 % of acetic acid is metabolized in the muscle, heart, and kidney cells. Propionic acid is the main precursor of glycogenesis in the liver and is necessary for energy production in the body.

BVFAs have harmful effects on the epithelial cells of the intestinal mucosa[48]. The fatty acids isobutyric and isovaleric induce apoptosis in the cells of the intestinal epithelium[49]; likewise, they can affect the exchange of ions in the colon and act as regulators in the absorption of sodium ions in the colon[50].

The increase in UBVFAs in the animals on Sb was likely due to the presence of β-glucans, mannan-proteins, and chitin in the cell wall of *S. boulardii*[51], which serve as excellent substrates for microbial fermentation[52] once the yeasts die in the intestine. Both yeasts present β-glucans in their cell wall; nevertheless, the cell wall is thicker in *S. boulardii* than in *S. cerevisiae*. The β-glucans of the cell wall of *S. cerevisiae* are more complex and insoluble in water[53], and it is likely that, due to this higher structural complexity and insolubility of the cell wall of *S. cerevisiae*, they cause lower bacterial fermentation, explaining why the animals that consumed this yeast in their diet had lower concentration of total VFAs compared to those of *S. boulardii*.

Antibiotics can eliminate harmful bacteria in the gut, reducing the presence of markers of inflammation[8] and restoring the intestinal barrier function[10]. Nonetheless, the negative side effect of using antibiotics in the feeds on the gut microbiome is the reduction of the gut bacterial diversity[28] and the destruction of beneficial bacteria due to the limited selectivity and the formation of bacterial strains insensitive to one or even several antibiotics due to the cross-resistance[27,54].

Among the different mechanisms of subtherapeutic antibiotics to improve piglet growth is the impact on the intestinal microbial ecosystem, decreasing the presence of pathogenic bacteria[55]. Due to this probable decrease in microbial populations, the animals that consumed the antibiotic diet presented the lowest values of UBVFAs and TVFAs, likely due to a decrease in microbial fermentation due to the

bacteriostatic effect of the antibiotic on the intestinal microbiota[56]. This is corroborated by other results[57]. Other authors reported that antibiotics decrease intestinal bacterial fermentation, modifying the profile of short-chain fatty acids[58,59].

The changes in the pH of the cecum and colon digesta and in the fermentation profile in the colon due to the diet consumed observed in the present work suggest modifications in the bacterial profile. The amount and composition of the volatile fatty acids produced in the large intestine depend on the amount and constitution of the resident microbiota[60]. There was an improvement in the intestinal health of newly weaned piglets that consumed a diet supplemented with *S. boulardii* through the promotion of the growth of beneficial bacteria and their metabolites formed during fermentation[61].

The possible prebiotic action of the dead yeasts in the gastrointestinal tract may contribute to the change in the gut microbiota and its relationship with UBVFAs[35]. The supplementation of a hydrolysate of *S. cerevisiae* walls in the diet of the weaned piglets favored growth rate and the immune function and activated the beneficial bacterial growth of *Lactobacillus* sp., demonstrating its prebiotic effect[62]. In a colonic simulation model, it was demonstrated that *S. cerevisiae* improved dry matter digestibility, probably because of an effect associated with a change in the bacterial population and the fermentation profile observed[63]. In piglets exposed to *Salmonella typhimurium* and supplementing the diet with yeast fermentation products containing bioactive compounds, there was[64] an increase in the diversity of gut microbiota, the abundance of butyrate-producing bacteria, and the concentrations of short-chain fatty acids.

## Conclusions and implications

The similarities observed between *S. cerevisiae* and *S. boulardii* for the weight of the digestive organs, the enzymatic activity, and the pH of the gastrointestinal contents show the possibility of their indistinct use in the diets of newly weaned piglets; however, *S. boulardii* showed advantages in the profile of the volatile fatty acids produced, which may have an interesting effect on the control of diarrheas. The acidification of the colonic environment and the high fermentative capacity of the two yeasts is highlighted. The results obtained suggest that these yeasts can be named functional feeds with a probable alternative use to antibiotics.

## Acknowledgments and conflicts of interest

The authors are grateful to the Autonomous University of Querétaro and the Animal Nutrition Laboratory of the Faculty of Natural Sciences of the UAQ for the funding provided to this project.

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